DIFFERENCES IN AMINO ACID COMPOSITION OF β-LACTOGLOBULINS A AND I

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The genetically different crystalline β-lactoglobulins A and B, discovered by Aschaffenburg and Drewry (1955, 1957a), have been demonstrated by physicochemical methods to be closely related chemically (Ogston and Tombs, 1957; Timasheff and Townend, 1960; Tanford and Nozaki, 1959). The electrophoretic measurements of Timasheff and Townend and the titration curve determinations of Tanford and Nozaki have indicated that β-lactoglobulin A has two more carboxyl groups than the B form. Additional evidence for a difference in primary structure has been provided by the "hybridization" experiments of Townend, Kiddy and Timasheff (1961). We have found direct confirmation of the difference in carboxyl groups and of other differences in composition by amino acid analyses, using an automatic recording apparatus as described by Spackman, Stein and Moore (1958).

The analyses were carried out on β-lactoglobulin crystallized from mixed herd milk by Palmer's method and on the A and B forms crystallized from typed milks according to Aschaffenburg and Drewry (1957b). We are indebted to R. Townend, of this Division, for making the typed milks available to us. Each protein was recrystallized at least three times before final dialysis and lyophilization.

The proteins were hydrolyzed at 110° for 24 hours in 6 N HCl in sealed, evacuated tubes. Three hydrolyzates each of mixed β-lactoglobulin (AB) and of the B form, and two of the A form were analyzed on the 150 cm. columns. Only

a single hydrolyzate of each was run on the 15 cm. column; but the high reproducibility of analyses, which is a characteristic of the automatic apparatus, can be illustrated by the results of these three runs, shown in Table I. The values are percentages of dry protein.

TABLE I

	AB	A	B
Lysine	11.7	11.9	11.7
Histidine	1.55	1.58	1.56
Ammonia	1.27	1.27	1.27
Arginine	2.70	2.72	2.71

The results of the 150 cm. column experiments also showed excellent agreement, not only between duplicate hydrolyzates but also for the majority of amino acids in the three proteins. Differences believed to be significant are presented in Table II. The figures are average percentages.

TABLE II

	AB	A	B
Aspartic Acid	11.2	11.4	10.6
Glycine	1.38	1.24	1.54
Alanine	6.94	6.67	7.00
Valine	5.80	5.92	5.39

The differences in aspartic acid and glycine content of A and B are undoubtedly significant. The small difference in alanine content must be verified by additional analyses, while that in valine content is subject to correction because longer periods of hydrolysis are usually required for maximal yields of this amino acid. In terms of amino acid residues per 35,000 molecular weight, the values in Table II show that β-lactoglobulins A and B differ by 2.0 aspartic acid, 1.4 glycine, 1.3 alanine and 1.6 valine residues. It is not yet possible to determine with certainty the precise number of residues in each form. Incidentally, the present analyses of β-lactoglobulin AB agree very well with the

classical analyses of Brand et al. (1945) and the more recent analyses of Stein and Moore (1949) by starch chromatography.

In preliminary experiments R. Townend and V. M. Ingram have shown by high voltage electrophoresis of tryptic digests of β-lactoglobulins A and B that differences exist in the patterns of peptides yielded by this technique. Similar results have been obtained by E. B. Kalan and R. Townend with chymotryptic digests. Further experiments are in progress in order to relate these differences with the differences in amino acid composition herein reported.

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